Bone Marrow Transplantation (2010) 45, 580–583

www.nature.com/bmt

LETTER TO THE EDITOR

Severe pulmonary toxoplasmosis after allo-SCT in two patients: from Toxoplasma genotyping to clinical management

Bone Marrow Transplantation (2010) **45,** 580–583; doi:10.1038/bmt.2009.167; published online 13 July 2009

Toxoplasma gondii infection can be a devastating disease in immunodeficient individuals. In these patients (AIDS, organ transplantation), severe toxoplasmosis can result mainly from reactivation of a latent infection. It can induce symptoms restricted to the central nervous system, lung, heart and eyes or cause disseminated infection.[1] Although severe toxoplasmosis is a rare but often fatal event, disease severity and outcome might be influenced by parasite genotype.[2] We report two cases of toxoplasmosis in haematopoietic SCT (HSCT; allo-SCT) patients, associated with non-type II strains of *T. gondii*. Susceptibility to toxoplasmosis of such non-HIV-immunocompromised patients is discussed in terms of underlying disease, toxoplasmosis prevention, clinical features, undergoing treatment and the potential role of the *T. gondii* genotype.

Case 1

A 15-year-old girl with the myelodysplastic syndrome RAEB underwent unrelated cord blood graft with HLA-DRB1 mismatch. At transplant, she was in remission after induction-type chemotherapy. Myeloablative conditioning consisted of BU, CY and anti-thymoglobulin. GVHD prophylaxis was provided with CYA and i.v. corticotherapy. During the neutropenic phase, she received broad-spectrum antibiotics (ceftazidime, vancomycin and metronidazole). Her pretransplantation serological test was positive for *T. gondii* (Table 1). On day 35 after transplantation, she developed fever and interstitial lung disease, which improved with cotrimoxazole and roxithromycin treatment until day 53, when the treatment was discontinued for liver toxicity. Engraftment was documented on day 45. On day 40, CMV infection was diagnosed and treated with ganciclovir followed by foscarnet. The patient developed acute respiratory failure, deteriorated rapidly in spite of intensive supportive therapies, and she became confused on day 54. The brain computed tomography scan showed two hypodense lesions in the right frontal region; *Toxoplasma* DNA was detected in a blood sample on day 67, and intracellular parasites were identified in MRC5 fibroblast blood culture. The patient died on the same day. Bacterial, fungal and viral cultures were negative. Postmortem analysis of bronchoalveolar lavage, cerebrospinal fluids and pleural sample revealed positive detection of *Toxoplasma* DNA in lung specimens and blood samples (Table 1), in agreement with pulmonary toxoplasmosis disseminated secondarily. Direct genotyping at six *T. gondii* microsatellite loci (*TUB2*, *TgM-A*, *W35*, *B17*, *B18* and *M33*) was done using multiplex PCR assay from bronchoalveolar lavage and blood samples. An atypical (recombinant) genotype was found (isolate referenced as LIL-2000-BRI).[2]

Case 2

A 40-year-old man underwent allo-SCT transplantation from an HLA-identical sibling donor for refractory CLL. Earlier lines of treatment included fludarabine, CY, doxorubicin, VCR and steroids. Reduced-intensity conditioning consisted of fludarabine plus 2 Gy TBI; GVHD prophylaxis was provided with CYA and mycophenolate mofetil. Engraftment was documented on day 25, after a pulmonary complication treated first with cotrimoxazole, later replaced with atovaquone (to manage an episode of acute cotrimoxazole-related nephrotoxicity). Pretransplantation serological analyses revealed a *Toxoplasma*-seronegative donor to a seropositive recipient (D−/R+) mismatch, the situation at maximum risk for toxoplasmosis reactivation (Table 2). At 4 months later (day +112), severe cutaneous GVHD (grade III–IV) occurred, requiring intense immunosuppression with methylprednisolone and anti-IL-2 receptor antibodies. On day 142, he developed haemolytic uraemic syndrome and deteriorated rapidly in spite of intensive supportive therapies. On day 145, he developed acute respiratory failure with severe hypoxaemia. The computed tomography scan showed pulmonary interstitial infiltrates, and nodules were seen in both lungs. Microbiological tests for virus and fungus detection were negative. Tachyzoites suggestive of *T. gondii* were identified on direct examination of Giemsa-stained bronchoalveolar lavage samples, confirmed by positive detection of *Toxoplasma* DNA using PCR (Table 1). Despite an anti-*Toxoplasma* treatment combining pyrimethamine and clindamycin, the patient died on day 150. Postmortem analysis of patient sera confirmed toxoplasmosis reactivation (Table 1). Direct *T. gondii* genotyping identified genotype III (isolate referenced as LIL-2003-LAM).[2]

In immunocompromised patients, toxoplasmosis is a life-threatening opportunistic infection, resulting mainly from reactivation.[1–4] *Toxoplasma*-seropositive allo-SCT recipients (in particular cord blood transplantation, such as in case 1) are particularly at risk, with a higher risk when recipients receive cells from a *Toxoplasma*-seronegative donor. Developing GVHD is also a risk factor, probably due to deep and long-lasting immunosuppressive therapy,[1] as in case 2.

Table 1 Two cases of disseminated toxoplasmosis in HCST patients: laboratory data

Sample	*Date (day)*	*Anti-Toxoplasma Ab detection*[a]			*Parasite detection*	
		ELISA IgG (UI/ml) (IgG avidity)	*ELISA IgM*	*ISAGA IgM—IgA*	*PCR*	*Cell culture*
Patient 1						
Blood	Pretransplantation	86	Negative	Negative	ND	ND
Pleural fluid	51				Positive	ND
BAL	52				Positive	ND
BAL	54				Positive	ND
CSF	54	3.5	Negative	Negative	Negative	ND
BAL	55				Positive	ND
Blood	57	4.6	Negative	Negative	Positive[b]	Positive
Blood	67	22	Negative	Negative	Positive	Positive
Patient 2						
Blood	Pretransplantation	9	Negative	Negative	ND	ND
Blood	7	7	Negative	Negative	ND	ND
Blood	35	<4	Negative	Negative	ND	ND
Blood	67	5	Negative	Negative	ND	ND
Blood	93	16 (High avidity)	Negative	Negative	ND	ND
Blood	115	20	Negative	Negative	ND	ND
Blood	127	8.5	Negative	Negative	ND	ND
Blood	132	9.2	Negative	Negative	ND	ND
BAL	148	<4	Negative	Negative	Positive[c]	ND

Abbreviations: BAL = bronchoalveolar lavage; CSF = cerebrospinal fluid; ND = not done.
[a]Anti-*T. gondii* Abs were determined by ELISA for IgG and IgM (Enzygnost IgG and IgM; Behring, Marburg, Germany) and by ISAGA for IgM and IgA (Department of Parasitology, Lille, France).
[b]Detection of *Toxoplasma* DNA (PCR assay based on B1 gene amplification) was performed postmortem, except for this sample.
[c]The positive PCR result was associated with microscopic detection of *T. gondii* tachyzoites on methanol–Giemsa-stained BAL smears.

Table 2 Toxoplasmosis risk management at Lille-2 University Hospital: follow-up of HSCT recipients during a 4-year period

	R/D serological status[a]			
	R+/D+	*R+/D−*	*R−/D+*	*R−/D−*
Clinical data				
Absence of GVHD	8 (40.0%)[b]	9 (47.4%)[c]	10 (58.8%)	5 (55.6%)
Existence of GVHD	12 (60.0%) (2 grade I, 6 grade III, 1 chronic GVHD, 4 unspecified grade)	10 (52.6%) (3 grade II, 2 grade III, 1 grade IV, 1 chronic GVHD, 3 unspecified grade)	7 (41.2%) (1 grade I, 3 grade II, 1 grade III, 1 grade IV, 1 chronic GVHD)	4 (44.4%) (2 grade II, 1 grade III, 1 chronic GVHD)
Anti-Toxoplasma chemoprophylaxis				
Co-trimoxazole	2 (11.1%)	1 (5.6%)	6 (42.9%) (1 grade III GVHD)	7 (100%)
Sulfadoxine + pyrimethamine	14 (77.8%)	16 (88.9%)	6 (42.9%) (1 grade I, 2 grade II, 1 chronic GVHD)	0
Pyrimethamine	1 (5.6%)	0	0	0
Spiramycin	1 (5.6%)	0	0	0
Atovaquone	0	1 (5.6%)	2 (14.2%) (1 grade II, 1 grade IV GVHD)	0
Untreated patients	2	1	3	2
Total		65		

Abbreviation: HSCT = haematopoietic SCT.
[a]R+ = *T. gondii*-seropositive recipient, R− = *T. gondii*-seronegative recipient D+ = *T. gondii*-seropositive donor; D− = *T. gondii*-seronegative donor.
[b]No. of patients expressed as percentage of the whole population in brackets.
[c]No. of patients.

Although meningoencephalitis has been described as the most common clinical presentation of toxoplasmosis in both AIDS patients and non-HIV-immunocompromised patients, pulmonary localization is frequent and often entails a poor outcome, as well as multiple organ involvement.[1–5] A rise of IgG-specific antibodies associated

with a high IgG-avidity index (case 2, Table 1) usually indicates serological reactivation, the most frequent situation in the HSCT recipient. Radiological analysis (case 1) and microscopic or molecular *Toxoplasma* detection in clinical samples (cases 1 and 2) are required to differentiate asymptomatic reactivation from disease.[1,3–5] As toxoplasmosis usually arises within 2–3 months after transplantation, efficient assessment of infectious risk is required during this high-risk period to avoid postmortem diagnosis.[1,3–6]

Given the high rate of allo-SCT toxoplasmosis mortality, it is now recommended to first determine the *Toxoplasma* serological status of donors and recipients, and then to prevent toxoplasmosis by using chemoprophylaxis based more often on cotrimoxazole and, in some centres, on pyrimethamine–sulfadoxine.[1,7] When the allo-SCT recipient is at risk (R+), serological follow-up is recommended,[1,4] associated with PCR follow-up when the recipient cannot tolerate chemoprophylaxis during the high-risk period.[1] As starting the anti-*Toxoplasma* therapy is a major prognosis factor, the use of highly sensitive real-time PCR to detect early parasite DNA on blood or plasma samples is now recommended.[1,6] To discuss further toxoplasmosis risk management, we analyzed how this risk was managed in our HSCT center, documenting each allo-SCT recipient follow-up during a 4-year period (2004–2007) at the Lille University Hospital. We focused on the systematic serological screening of donor and recipient before transplantation aiming at identifying a R+/D− mismatch, anti-*Toxoplasma* chemoprophylaxis administered to the recipient, and whether GHVD was present (Table 2). Among the 80 follow-ups corresponding to 79 patients (one patient had two grafts), eight did not undergo transplantation, and seven who had incomplete follow-up were excluded. The following observations were made: (i) no other fatal toxoplasmosis occurred; (ii) among the 65 patients engrafted and followed up during the period, 39 (62%) were seropositive recipients (R+), and 19 (29%) represented the high-risk mismatch situation (R+/D−, Table 2). Positive recipient serological status clearly influenced the chemoprophylaxis protocol choice in the Lille University Hospital, as 88.9% of R+/D− and 77.8% of R+/D+ were treated using pyrimethamine+sulfadoxine combination (in association with folinic acid, as recommended). In contrast, the R−/D− subgroup (at low risk of primoinfection) received exclusively cotrimoxazole as first-line prophylaxis. Toxoplasmosis prophylaxis of the R−/D+ subgroup seems to take into account GVHD status, probably as an indirect indicator of intense immunosuppression. Although GVHD was found in similar proportion in each R/D subgroup (Table 2), patients in the R−/D+ subgroup who presented GVHD received pyrimethamine–sulfadoxine prophylaxis (in 66.7% cases), cotrimoxazole being preferentially administrated to R−/D+ without GVHD (83.3%, Table 2). (iii) Atovaquone was used in three cases as an alternative recommended drug when intolerance to sulphonamide/sulfone was present.[1] (iv) Eight patients did not receive anti-*Toxoplasma* chemoprophylaxis (Table 2), corresponding to R−/D− cases ($n=2$), for whom primoinfection is prevented essentially by hygiene and diet measures, and to R−/D+ ($n=3$) for whom the risk of contracting *Toxoplasma* infection at the time of HSCT is rare.[5] The last three cases progressed to death within the 30 days after transplantation, a period corresponding to the delay recommended before prophylaxis initiation to minimize haematological adverse effects.[1]

Our local experience in toxoplasmosis risk management seems to be indicative of the situation of developed countries, with the exception of using pyrimethamine–sulfadoxine chemoprophylaxis, which is restricted to some centres including the Lille University Hospital, instead of using cotrimoxazole, which is the most widely used drug in the US and European HSCT centres.[1] The molecular diagnosis was also modified at our center: PCR follow-up is now recommended during the high-risk period to anticipate toxoplasmosis diagnosis and prevent a fatal outcome.

The poor outcome of toxoplasmosis in allo-SCT recipients is consistent with the major immunosuppression status of patients that might be responsible for the extreme severity of toxoplasmosis and its ability to disseminate quickly. Besides these well-known elements, *Toxoplasma* genotypes have been proposed to influence clinical presentation and outcome.[8]

The *Toxoplasma* population structure is composed of three clonal lineages: types I, II and III, predominating in North American and European populations, whereas South American strains are more polymorphic.[9] Although type II is the most common genotype isolated in Europe,[2,9] genotyping performed on our isolates identified an atypical *Toxoplasma* strain in case 1 and a type III in case 2. Atypical genotypes are known to be virulent in mice[9] and have been described in severe toxoplasmosis.[8] As they are characterized by an allele combination mostly between types I and III, and are associated with the *Toxoplasma* cycle involving wild felines usually circulating in South America,[2,8,9] details concerning the geographical origin of our patients, the existence of a stay in a tropical region, as well as patients' dietary habits (especially any consumption of game) were documented retrospectively as negative. In patients, the links between *Toxoplasma* genotype and severity is still a matter of debate, especially when the host is immunocompromised.[2] Additional studies are currently needed to better understand the relation between genotypes and toxoplasmosis, especially to access the ability to develop disseminated toxoplasmosis in immunocompromised patients infected with a non-type II strain. If this ability is demonstrated, toxoplasmosis risk management should take into account the parasite's genotype when patients such as allo-SCT recipients are evaluated for toxoplasmosis risk.

In conclusion, identifying immunocompromised patients at high risk of toxoplasmosis (mismatch patients), to prevent severe toxoplasmosis by establishing serological and molecular follow-up and prescribing chemoprophylaxis remains the current recommended toxoplasmosis prevention strategy. The role of parasite genotype within the host–parasite interaction and disease severity is still being debated and should be evaluated in a multicenter prospective study using new serological screening.[10]

Conflict of interest

The authors declare no conflict of interest.

L Delhaes[1], J-C Mraz[1], E Fréalle[1], I Durand-Joly[1], L Magro[2], D Ajzenberg[3,4], M-L Dardé[3,4], E Dei-Cas[1] and I Yakoub-Agha[2]

[1]*Parasitology–Mycology Department—EA3609—Laboratoire associé du Centre National de Référence Toxoplasmose, Lille Hospital—Faculty of Medicine, Lille 2 University, Lille, France;*
[2]*Service des Maladies du Sang, Lille Hospital—Faculty of Medicine, Lille 2 University, Lille, France;*
[3]*Centre National de Référence Toxoplasmose/Toxoplasma Biological Resource Center, Centre Hospitalier-Universitaire Dupuytren, Limoges, France and*
[4]*Laboratoire de Parasitologie-Mycologie, EA 3174-NETEC, Faculté de Médecine, Université de Limoges, Limoges, France*
E-mail: l-delhaes@chru-lille.fr

References

1 Derouin F, Pelloux H, ESCMID Study Group on Clinical Parasitology. Prevention of toxoplasmosis in transplant patients. *Clin Microbiol Infect* 2008; **14**: 1089–1101.
2 Ajzenberg D, Yera H, Marty P, Paris L, Dalle F, Menotti J *et al*. Genotype of 88 *Toxoplasma gondii* isolates associated with toxoplasmosis in immunocompromised patients, and correlation with clinical findings. *J Infect Dis* 2009; **199**: 1155–1167.
3 Goebel WS, Conway JH, Faught P, Vakili ST, Haut PR. Disseminated toxoplasmosis resulting in graft failure in a cord blood stem cell transplant recipient. *Pediatr Blood Cancer* 2007; **48**: 222–226.
4 Longoni DV, Fumagalli R, Fumagalli M, Cappellini A, Uderzo C. Severe disseminated toxoplasmosis after unrelated bone marrow transplantation: a case report. *Haematologica* 2000; **85**: 781–782.
5 Chandrasekar PH, Momin F. Disseminated toxoplasmosis in marrow recipients: a report of three cases and a review of the literature. Bone Marrow Transplant Team. *Bone Marrow Transplant* 1997; **19**: 685–689.
6 Vaessen N, Verweij JJ, Spijkerman IJ, van Hoek B, van Lieshout L. Fatal disseminated toxoplasmosis after liver transplantation: improved and early diagnosis by PCR. *Neth J Med* 2007; **65**: 222–223.
7 Foot AB, Garin YJ, Ribaud P, Devergie A, Derouin F, Gluckman E. Prophylaxis of toxoplasmosis infection with pyrimethamine/sulfadoxine (Fansidar) in bone marrow transplant recipients. *Bone Marrow Transplant* 1994; **14**: 241–245.
8 Carme B, Bissuel F, Ajzenberg D, Bouyne R, Aznar C, Demar M *et al*. Severe acquired toxoplasmosis in immunocompetent adult patients in French Guiana. *J Clin Microbiol* 2002; **40**: 4037–4044.
9 Khan A, Fux B, Su C, Dubey JP, Darde ML, Ajioka JW *et al*. Recent transcontinental sweep of *Toxoplasma gondii* driven by a single monomorphic chromosome. *Proc Natl Acad Sci USA* 2007; **104**: 14872–14877.
10 Sousa S, Ajzenberg D, Vilanova M, Costa J, Dardé ML. Use of GRA6-derived synthetic polymorphic peptides in an immunoenzymatic assay to serotype *Toxoplasma gondii* in human serum samples collected from three continents. *Clin Vaccine Immunol* 2008; **15**: 1380–1386.